EXHIBIT 16

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The Fetal Valproate Syndrome

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We evaluated seven children who had been exposed to sodium valproate (or valproic acid) in utero. A consistent facial phenotype was observed in all seven in addition to other birth defects in four. The facial changes consisted of epicanthal folds which continued inferiorly and laterally to form a crease or groove just under the orbit, flat nasal bridge, small upturned nose, long upper lip with a relatively shallow philtrum, a thin upper vermillion border, and downturned angles of the mouth. Hypospadias, strabismus, and psychomotor delay were found in two males; two children had nystagmus and two had low birth weight.

Key words: valproate, valproic acid, fetal valproate syndrome, birth defect, epilepsy, strabismus, hypospadias, teratogen, disruption sequence

INTRODUCTION

Valproic, or dipropylacetic acid (VPA) and its salt, sodium valproate (Depakene®), are used to treat a wide variety of seizure disorders. Although first synthesized a century ago, interest in this substance as a drug did not begin until 1963 following the accidental discovery of its anticonvulsant properties while being used as a solvent to test other drugs [Pinder et al, 1977]. After completion of clinical trials, sodium valproate and valproic acid were released first in Europe and subsequently in the

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United States for the treatment of epilepsy in adults and children. An initially low reported incidence of side effects coupled with satisfactory clinical results led to widespread use. It has been estimated that between 700 and 1,000 pregnant women take valproic acid each year in the United States in spite of a clear statement on the package insert warning of potential risks of teratogenesis [Morbidity and Mortality Weekly Report, 1982]. Until recently no convincing evidence was presented demonstrating a teratogenic effect in humans, although several case reports associating a variety of birth defects with the drug were published [Dalens et al, 1980; Clay et al, 1981; Gomez, 1981; Thomas and Buchanan, 1981; Bailey et al, 1983; DiLiberti, 1983; Hanson et al, 1984]. However, recent reports also suggest an association between maternal VPA therapy and neural tube defects [Robert and Guibaud, 1982; Bjerkedal et al, 1982; Jeavons, 1982].

During the last 3 years we have evaluated and followed seven children exposed to VPA or sodium valproate in utero. Similarities among these children suggest that, even in the absence of major teratogenic effects, a consistent craniofacial appearance, the fetal valproate syndrome (FVS), results from intrauterine exposure to this drug.

METHODS

Four of the children were identified by asking local neurologists, pediatricians, and clinics to refer infants with histories of intrauterine exposure to valproic acid for evaluation. None of these four were considered to have developmental or medical problems prior to the time of the evaluation. The other three children had each been referred for evaluation because of developmental delay or birth defects. During the period of this study no other children exposed to valproic acid were seen.

CLINICAL REPORTS

Patient 1

Patient 1, a 3-year-old girl, was born to a gravida 3, para 2, 21-year-old mother after a 36 week pregnancy which was uncomplicated except for maternal anticonvulsant treatment. Birth weight was 2,660 g. Mild hypocalcemia and hypoglycemia were present briefly during the first 48 hours of life. She was breast fed and had no other neonatal problems except for irritability.

Her mother began having generalized seizures at 11 years and was initially treated with phenobarbital. Subsequently, clinical and electroencephalographic evidence of petit mal epilepsy became evident and valproic acid therapy was begun just before conception of this child. During this pregnancy the prescribed dose of valproic acid initially was 500 mg daily and subsequently raised to 750 mg during the third trimester. Blood levels were not done during the pregnancy but were in the therapeutic range shortly afterward. Other drug exposure included occasional acetaminophen and infrequent, modest alcohol consumption. The mother had no generalized seizures during pregnancy.

The infant was unremarkable except for facial appearance (Fig. 1A,B). She had slight upward slant of the palpebral fissures and bilateral epicanthal folds which continued inferiorly and laterally to form a fairly well-defined crease or groove along the inferior margin of the orbit. A flat nasal bridge, small nose with anteverted nostrils, long upper lip with a relatively shallow philtrum, and a thin, downturned

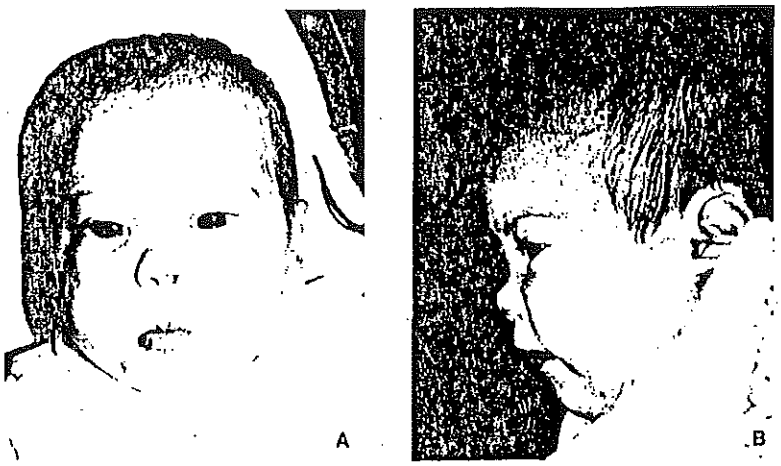

Fig. 1. A,B) Patient 1 at 7½ months.

upper vermillion border also present. Her ears were small with incomplete development of the lobules and posterior rotation (Fig. 1B). She has been followed for nearly 3 years and her health, growth (height 94 cm, weight 13.6 kg, head circumference 49.9 cm), and development all appear to be normal.

Patient 2

Patient 2, a boy, was born following an uneventful pregnancy, labor, and delivery to a 34-year-old gravida 1 mother. His birth weight was 3,770 g, length 53 cm, and head circumference 34 cm. The neonatal course was complicated by transient jitteriness and feeding problems.

His mother started having seizures during adolescence and currently has petit mal seizures almost daily. She has been treated with valproic acid, 1,000 mg per day and mephobarbital 100 mg per day and received these amounts throughout the pregnancy. She had no exposure to other drugs, including alcohol, during the pregnancy.

The infant was unremarkable except for a small area of cutis aplasia of the scalp and craniofacial changes which included a flat nasal bridge, small epicanthal folds extending into infraorbital creases bilaterally, small upturned nose, long upper lip with relatively shallow philtrum, and a downturned upper lip with a thin vermillion border (Fig. 2). At 2 years growth and development were normal.

Patient 3

Patient 3, a girl, was born at 41 weeks to a 26-year-old gravida 1 mother from a breech presentation. Birth weight was 3,020 g and the neonatal course was unremarkable.

Her mother began having seizures after a head injury suffered in an automobile accident several years previously. She had been treated with diphenylhydantoin but was switched to valproic acid before conception. She took 500 mg daily throughout

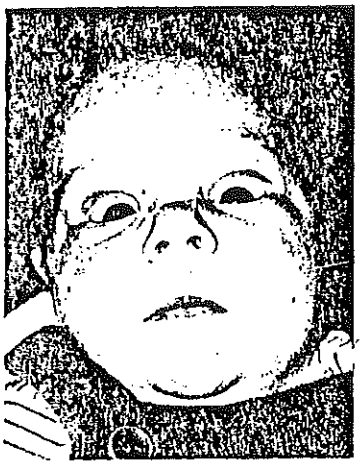

Fig. 2. Patient 2 at 1 year.

Fig. 3. Patient 3 at 3 months.

the pregnancy. Her blood levels were monitored and found to be therapeutic. She took no other drugs or alcohol during the pregnancy.

The infant was unremarkable except for facial changes which included small epicanthal folds with infraorbital creases, mild flatness of the nasal bridge, slightly long upper lip and shallow philtrum, downturned angles of the mouth with thin vermillion borders (Fig. 3), and a small subungual capillary hemangioma. At 2 years growth (height 78.9 cm, weight 10.2 kg, head circumference 47 cm) and development were normal and she remains in good health.

Patient 4

Patient 4, an infant boy, is a brother of patient 3. He was born normally at 38 weeks by repeat caesarean section. Birth weight was 2,480 g. The neonatal course was reported to be normal. His mother took 750 mg of valproic acid and 1,000 mg of carbamazepine daily throughout the pregnancy. Blood levels of both drugs were monitored regularly.

The infant was normal except for facial changes which included epicanthal folds with infraorbital creases, a slightly upturned nose, long upper lip, and downturned angles of the mouth with relatively thin upper vermillion border (Fig. 4). The ears were anteverted and the right was also slightly anteflexed.

Patient 5

Patient 5 was an infant boy whose mother took valproic acid, diphenylhydantoin, phenobarbital, and carbamazepine during the entire pregnancy. She had seizures and inadequate nutrition for several weeks as a consequence of noncompliance with this drug regimen. Patient 5's birth weight was 3,300 g and during the neonatal period he had feeding difficulties and inadequate weight gain.

At 3 1/2 months, he had a small head (39 cm = 3rd centile) with prominent metopic suture, strabismus, fine horizontal nystagmus, epicanthal folds with infraor-

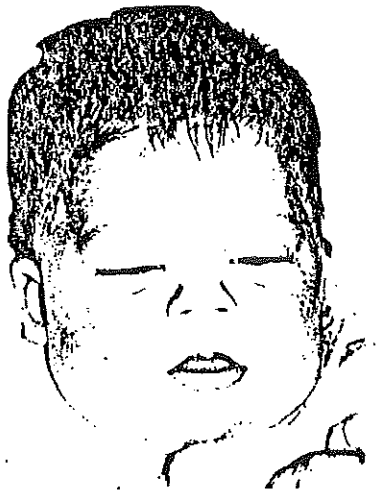

Fig. 4. Patient 4 at 2½ months.

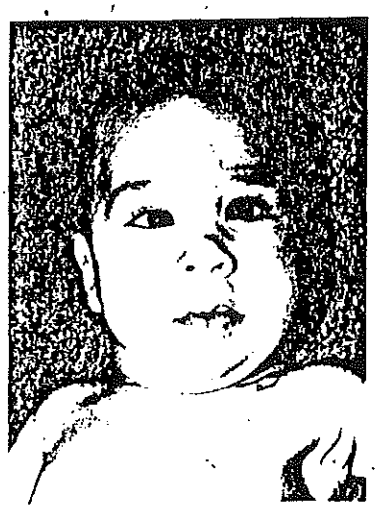

Fig. 5. Patient 5 at 3½ months.

bital creases, flat nasal bridge, anteverted nostrils, long upper lip with a shallow philtrum, posteriorly angulated ears (Fig. 5), and first degree hypospadias with chordee. His psychomotor development appeared to be delayed.

Patient 6

Patient 6 was an infant boy born at 38 weeks to a 20-year-old gravida 1 mother. The pregnancy was complicated by toxemia with reduced fetal growth rate; birth weight was 1,700 g and head circumference 30.5 cm.

The mother had begun having generalized seizures approximately 4 months before conception. She was first treated with diphenylhydantoin, then phenobarbital, and finally sodium valproate just prior to conception. Her initial valproate dose was 600 mg daily but this was increased to 2,000 mg in the first month and 2,400 mg daily by the third month of gestation.

The following abnormalities were noted at birth: single umbilical artery, penoscrotal hypospadias with chordee, flexion contractures of the fingers with a missing flexion crease on the fifth fingers and a transverse palmar crease on the left hand, calcaneovalgus deformity of the feet with a convex inferomedial border and partial syndactyly of the first and second toes. Craniofacial characteristics included large, apparently low-set and posteriorly angulated ears, esotropia, a small nose with anteverted nostrils, a long upper lip with relatively shallow downturned angles of the mouth, a thin vermillion border of the upper lip, short palpebral fissures, and epicanthal folds (Fig. 6). A karyotype was obtained and found to be 46 XY, with no observable abnormalities.

Inadequate suck necessitated gavage feeding for 3 weeks; afterward he had failure to thrive, congestive heart failure owing to patent ductus arteriosus, intermittent esotropia, and bilateral inguinal hernias. At 8 months a developmental assessment revealed a functional level of 3–4 months.

Fig. 6. Patient 6 at 10 months.

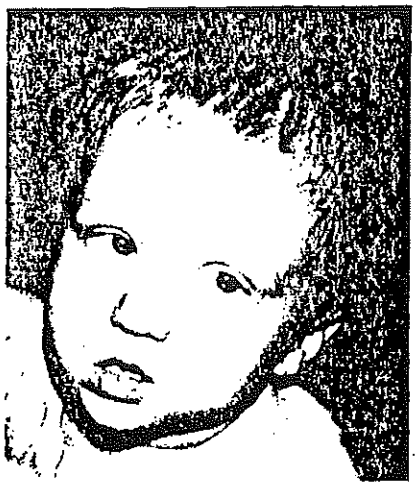

Fig. 7. Patient 7 at 3 years.

Patient 7

Patient was a boy born to a 20-year-old gravida 2, para 1 mother by caesarean section at 40 weeks. Birth weight was 4,250 g, length 54.6 cm, and head circumference 36 cm. His mother took 2,250 mg of valproic acid daily throughout the entire pregnancy but was exposed to no other drugs or alcohol.

At 3 years he had delayed psychomotor development. Facial changes included bilateral epicanthal folds, posteriorly angulated ears, and a relatively small mouth with a thin upper vermillion border (Fig. 7). He has had several generalized seizures and has continuous pendular nystagmus. Height (94 cm), weight (14.1 kg), and head circumference (50.5 cm) were all in the normal range.

DISCUSSION

These seven children exposed to VPA or sodium valproate in utero all appear to have a consistent facial phenotype characterized by epicanthal folds connecting with an infraorbital crease or groove, a flat nasal bridge, a small nose with anteverted nostrils, a long upper lip with relatively shallow philtrum, a relatively small mouth with downturned angles, and a thin upper vermillion border. Several previous reports have suggested that an abnormal facial appearance might result from intrauterine exposure to valproic acid [Dalens et al, 1980; Clay et al, 1981; Thomas and Buchanan, 1981; Nau et al, 1981; Jeavons, 1982]. The descriptions of the faces in these cases are suggestive of the findings in our patients (Table I) but unfortunately no clinical photographs have been published, making it difficult to confirm this impression. The children with FVS demonstrate some of the same craniofacial characteristics that have been reported in the fetal hydantoin syndrome (FHS) but appear to differ in several ways [Smith, 1982]. Children with FHS tend to have hypertelorism, which is not found in any of our patients. The nasal bridge is correspondingly broad in FHS but not appreciably widened in FVS, and the bifrontal diameter of the skull appears to be broad in FHS and narrow in FVS. We have not seen an infraorbital crease or

TABLE 1. Descriptions of Facial Appearance in Published Cases of Intrauterine Valproate Exposure

Dalens et al [1980]	"hypoplastic root of nose" "hypoplastic fronto-orbital edges" "low implantation of ears"
Thomas and Buchanan [1981]	"depressed nasal bridge"
Clay et al [1981]	"coarsened facies" "prominent forehead" "flat nasal bridge" "small nose"
Nau et al [1981]	"odd facial appearance"
Jeavons [1982]	"dysmorphic"
Koch et al [1983]	"small nose" "microstomy" "thin lips" "low-set and rotated ears"

groove, which appears to be common in FVS, in any of our own or in published photographs of patients with the FHS. The mouth, which tends to be widened in FHS, seems to be relatively small in FVS. Four children had mild to moderately abnormal external ears—an abnormality that has been found in several previous cases [Dalens et al, 1980; Koch et al, 1983].

In addition to the craniofacial changes, other anomalies were found in several of the children. Because of the small number of cases we cannot be certain that any of them are consistently associated with FVS. Two patients (4 and 6) have hypospadias, strabismus, and developmental delay. These two boys bring the total number of reported cases of hypospadias to three and the total number of males with genitourinary tract malformations to four, suggesting a possible teratogenic effect of VPA on the genitourinary tract [Bailey et al, 1983]. Developmental delay has been noted in five other reported cases but few data on long-term psychomotor development are available [Nau et al, 1981; Clay et al, 1981]. Strabismus has apparently not been reported previously.

Two patients met the definition of low birth weight (less than 2,500 g) and a third was only slightly above this weight. Since seven previously reported children were either of low birth weight, premature, and/or small for gestational age, we suspect that VPA treatment may be associated with intrauterine growth problems. It is of course possible that the underlying seizure disorder is a major contributor to unsatisfactory obstetric outcome.

Although only one of our patients had a congenital heart defect (CHD), we are impressed with the number of cases of CHD reported in association with intrauterine VPA exposure [Thomas and Buchanan, 1981; Clay et al, 1981; Nau et al, 1981; Bailey et al, 1983; Robert and Rosa, 1983; Koch et al, 1983; Hanson et al, 1984]. A larger epidemiologic study will obviously be needed to confirm an association between CHD and FVS.

Considerable interest in an association between neural tube defects and VPA exposure has been expressed recently [Robert and Guibaud, 1982; Bjerkedal et al, 1982]. Although the validity of some study methods has been questioned, available

data suggest that this reported association may be real [MacRae, 1982; Morbidity and Mortality Weekly Report, 1982]. We have obtained lumbosacral spine radiographs on several of our patients and found no evidence of occult NTD.

We do not think that a multiple drug regimen during pregnancy has any important effect on our conclusions. Four of our patients were exposed to VPA or sodium valproate alone; one to VPA and carbamazepine; one to VPA and mephobarbital; and one to VPA, diphenylhydantoin, phenobarbital, and carbamazepine. Neither barbiturates nor carbamazepine have been reported to produce a consistent craniofacial phenotype. The facial changes were most pronounced in two children who received VPA alone (1 and 6) but were similar in all of the children, suggesting that VPA exposure was the common denominator. The diphenylhydantoin exposure in patient 5 could be a confounding factor in the interpretation of the fact but in our opinion this child looks far more like other children with FVS (particularly case 6) than like any children we have seen with FHS.

VPA teratogenicity has been demonstrated experimentally in rabbits, rats, and mice. Induced anomalies included renal agenesis, cleft palate, encephalocele, vertebral defects, rib fusion, and ablepharia [Pinder et al, 1977; Lancet (editorial), 1982]. These effects appeared to be dose related and were observed with doses several times higher than those given in clinical practice. Transplacental passage of VPA in humans has been demonstrated [Dickinson et al, 1979]. Small amounts are also present in human milk but milk levels are generally only several percent of the blood levels [Dickinson et al, 1979]. We have confirmed this in one of our patients. Thus, breast feeding is probably safe for the infant of a mother taking VPA.

Although VPA has been reported to produce severe, sometimes fatal liver disease in older children and adults, we found no convincing evidence of hepatic dysfunction in any of our patients. Patient 1 had hypoglycemia and hypocalcemia, which could conceivably have been due to liver disease but was more likely a result of the relatively low birth weight and prematurity. Liver enzymes were subsequently normal in this child and one other tested infant. None of the children was reported to have had obvious jaundice.

We propose that intrauterine exposure to VPA produces a consistent craniofacial phenotype which may possibly be associated with other abnormalities including hypospadias, strabismus, psychomotor delay, low birth weight, congenital heart defects, nystagmus, and neural tube defects. Large epidemiologic studies should be carried out to confirm these associations and determine actual risks.

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